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Article

An Analysis of SARS-CoV-2 in Wastewater to Evaluate the Effectiveness of Nonpharmaceutical Interventions against COVID-19 in The Netherlands

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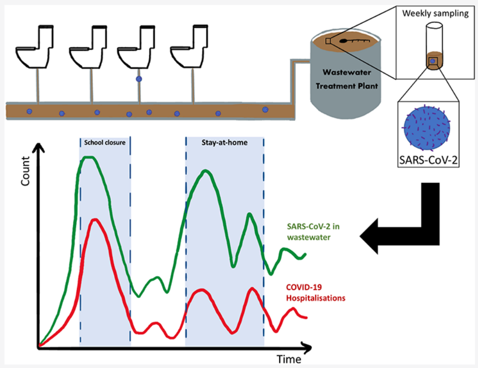
ABSTRACT: Wastewater-based epidemiology (WBE) is increasingly being recognized as a powerful tool for detecting and monitoring SARS-CoV-2 trends at a population level. This study looked to extend the use of WBE to explore the effectiveness of nonpharmaceutical interventions (NPIs) that have been used in response to COVID-19 and compare the results to the effect of such interventions on COVID-19 hospitalizations. A data-driven approach demonstrated that trends of SARS-CoV-2 RNA in wastewater, from Amsterdam and Utrecht (The Netherlands), precede hospitalizations by at least 3−9 days. Additionally, the effect of NPIs can be seen in wastewater and hospitalizations after 20 and 24 days, respectively. Changepoint analysis indicated that the closure of schools and universities significantly reduced the level of SARS-CoV-2 RNA in wastewater and COVID-19 hospitalizations. Regression modeling suggested the stay-at-home policy is an effective intervention for reducing the level of SARS-CoV-2 RNA in wastewater, whereas the closure of workplaces significantly reduced hospitalizations in both Dutch cities. This study demonstrates how WBE can be used to inform public health decisions and anticipate future strain on healthcare facilities in major cities but also indicates a need for higher temporal resolution of wastewater sampling.



KEYWORDS: *COVID-19, SARS-CoV-2, wastewater-based-epidemiology, nonpharmaceutical interventions, regression, changepoint, modeling*

■ INTRODUCTION

On January 31, 2020, the coronavirus disease (COVID-19) was declared a global health emergency.[1] Since then, there has been an ongoing global struggle to contain the spread of this disease. Given that vaccinations for COVID-19 were not widely available until 2021, most efforts to limit the spread of the virus came in the form of nonpharmaceutical interventions (NPIs), and in future, NPIs will likely still play an important role. Many countries adopted NPIs that aim to reduce human contact or identify and subsequently segregate infected individuals. However, NPIs are associated with high societal and economic costs. Investigations into the effectiveness of NPIs against SARS-CoV-2 can help determine which ones are essential to implement and which can be foregone in a bid to limit interruption to everyday life.

At present, most information about NPI effectiveness has come from national confirmed case data, death totals, and hospitalizations. Confirmed cases generally derive from RT-PCR testing,[2] which is per se a highly accurate approach and allows track-and-trace systems to identify people for isolation but does rely on testing capacity and willingness to get tested. Recent modeling studies that have used reported cases and deaths have produced conflicting results as to the effectiveness of interventions. Some studies suggest the stay-at-home order is the most successful at reducing SARS-CoV-2 prevalence, while others find the closure of schools and workplaces is more effective.[3,4]

Despite improvements in testing capacity, PCR testing can be a biased surveillance method. This can be due to seasonal events altering the number of swab tests dispensed weekly and reporting delay differing over time and regions.[5] Furthermore, PCR testing often misses asymptomatic COVID-19 cases, estimated to make up 17−20% of infections, and presymptomatic cases,[6,7] each of which allows transmission of the virus. Thus, there is increased uncertainty surrounding the estimated prevalence of SAR-CoV-2 from PCR-confirmed cases. Hospitalizations, as a measure, do not come with the same limitations as PCR testing because the admissions criteria have remained more consistent throughout the pandemic. Additionally, this measure can be used to determine the strain

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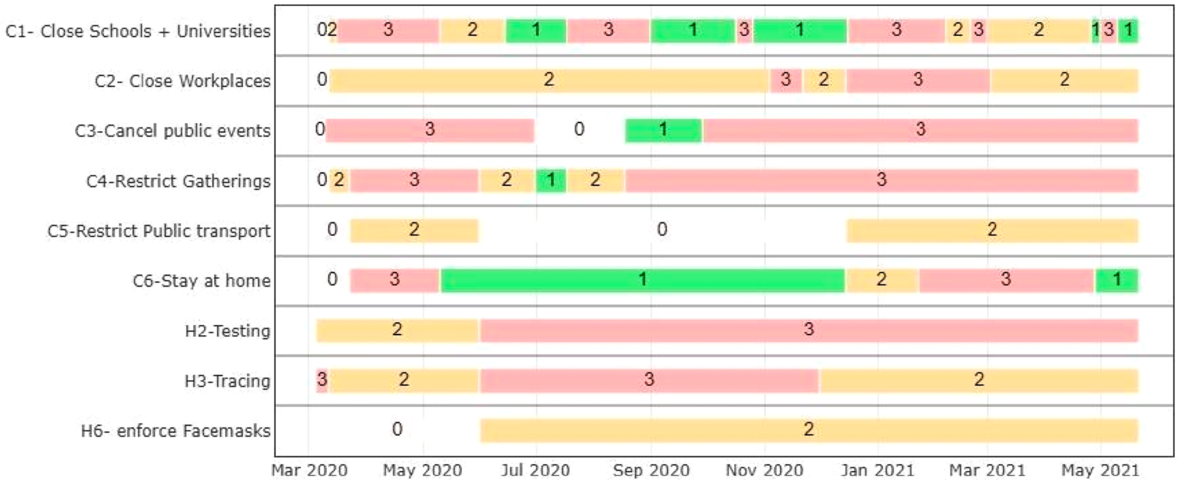

Figure 1. Timeline of COVID-19 nonpharmaceutical interventions (NPI's) considered in this analysis, between March 2020 and May 2021. Legend: 0, no action; 1, recommended intervention; 2, partial intervention; 3, full intervention.

placed on healthcare facilities. However, the delay between symptom onset and admission is generally greater than the period between symptom onset and a positive test, so this measure is not ideal for real-time assessment of COVID incidence.[8−11]

It has been suggested that wastewater-based epidemiology (WBE), which is increasingly being incorporated into national COVID-19 surveillance schemes,[12,13] is an effective method for monitoring the amount of SARS-CoV-2 circulating in cities.[13,14] A significant proportion of COVID-19 cases, including pre- and asymptomatic individuals, have been found to shed SARS-CoV-2 RNA in their stools (27.4—55.1% according to Parasa et al.[15]), and consequently, SARS-CoV-2 RNA can be detected in sewage samples.[16] Given that almost the entire population will use toilets, and that these are connected to centralized wastewater treatment plants (WWTPs) in developed countries, sewage samples taken at WWTPs can provide an accurate measure of the true circulation of the virus within a population. There is also evidence to suggest that sewage surveillance is sensitive enough to detect the occurrence of COVID-19 cases days before these are reported to or detected by the authorities. For example, SARS-CoV-2 RNA was detected in the wastewater of Amersfoort, The Netherlands, 6 days before the first COVID-19 cases were reported in the city.[16] Due to uncertainties in the SARS-CoV-2 RNA shedding rate,[17−19] it is at present difficult to quantify the number of infectious individuals. However, the SARS-CoV-2 concentration in wastewater has been used to indicate trends in COVID-19 circulation in major cities.[20,21]

Only a few intervention studies have currently used wastewater data to evaluate the effectiveness of NPIs throughout the COVID-19 pandemic.[22,23] Hillary et al.[22] discovered a negative correlation between SARS-CoV-2 gene copies and the time period after implementation of a national lockdown in March 2020 in five of six U.K. sites. In contrast, Wurtz et al.[23] found no significant correlation between the second lockdown and circulation of SARS-CoV-2 in Marseille's wastewater. However, these studies[22,23] were each carried out over independent time frames of fewer than 6 months, and with minimal interventions assessed, so little comparison can be made between studies.

Considering this, the study presented here aimed to utilize WBE data collected in two Dutch cities to investigate whether significant changes in SARS-CoV-2 circulation occurred in response to implementation or relaxation of individual NPIs. This study also aimed to determine the delay period between the implementation or relaxation of individual NPIs and respective changes in the SARS-CoV-2 concentration in wastewater. However, it is also important to know how well these results translated to hospital admissions and, thus, how wastewater surveillance can be used to help manage the strain on healthcare facilities. Therefore, these investigations were repeated using hospital admissions.

■ MATERIALS AND METHODS

Description of Data Sets. Daily positive COVID-19 clinical tests and hospitalizations in Utrecht and Amsterdam, The Netherlands, were retrieved from the Environmental Systems Research Institute (ESRI) NL COVID-19 Hub.[24] These data were extracted between March 2, 2020, and May 25, 2021. Both daily positive tests and daily hospitalizations were smoothed for analysis by applying a seven-day centered rolling average.

Wastewater data were sourced from the KWR Water Institute. Wastewater samples (i.e., 24 h flow-proportional composites) were collected once per week at the influent of the WWTPs serving the cities of Utrecht and Amsterdam. The populations served by the WWTPs in Utrecht and Amsterdam were taken from the database of the Central Bureau of Statistics[25] and were 267 886 and 669 401, respectively. RT-qPCR was used to quantify SARS-CoV-2 RNA in the form of N2 gene copies per milliliter of sewage water (GC/mL). The method used is identical to the one described in detail by Medema et al.[16] and involved the use of four primers, namely, the N1−N3 regions of the nucleocapsid (N) gene as well as the envelope protein (E) gene of SARS-CoV-2. F-Specific RNA phages were used, and reported recoveries of the purification and concentration steps were 73 ± 50% (n = 16).[16] The recovery efficiency of both RNA extraction and qRT-PCR of the method used was 30.4 ± 22.3% and was determined using Dengue virus as an internal control.[16] Rainfall, tourism, and commuters fluctuated during the pandemic, which disrupts estimates of the dilution or number

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of people contributing to the viral load in sewers. Therefore, quantification of human biomarker CrAssphage (GC/mL) from these WWTPs was also used to normalize the human fecal contribution in the wastewater, as previously done in WBE studies.[19,22] Quantification of CrAssphage was performed on duplicate nucleic acid extracts using the specific CrAssphage CPQ_064 qPCR method described by Stachler et al.[26] and recently implemented by Heijnen et al.[27] All RT-PCR analyses were run in duplicate, and the average is used here.

Details of the dates and level of NPI policies introduced in The Netherlands between March 5, 2020, and May 21, 2021, were obtained from the Oxford COVID-19 Government Response Tracker (OxCGRT) database.[28] These data were checked against the National Institute for Public Health and the Environment (RIVM) COVID-19 archives[29] to ensure consistency. From this database, the policies selected for this analysis are school and university closures (c1), closure of workplaces (c2), cancellation of public events (c3), gathering restrictions (c4), closure of public transport (c5), stay-at-home order (c6), testing policy (h2), tracing policy (h3), and facemask policy (h6). Public information campaigns were excluded because this policy remained consistent throughout the pandemic, according to the OxCGRT database, so changes in wastewater signal or hospitalizations could not be attributed to this policy. Additionally, adherence to internal and international travel restrictions is likely a culmination of the stay-at-home order and other policies, such as reduced public transport. Therefore, in an effort to prevent overestimation of significance, it is assumed that the stay-at-home order also accounts for travel restrictions.

Each of these NPIs, within the OxCGRT database, originally consisted of multiple levels of implementation; however, the leveling criteria were inconsistent across policies. To allow a fair comparison between NPIs, the levels of implementation were altered to no interventions (0), recommended intervention (1), partial intervention (2), and full intervention (3), as shown in Figure 1. c1 was adapted to include school holidays as a full intervention measure. c6 was adapted to include the period between December 15, 2020, and January 22, 2021, as a partial intervention given the strong advice to stay at home. The curfew period, introduced in January, was included as a full intervention within the stay-at-home policy.

Given that COVID-19 interventions are implemented and relaxed as a direct response to rising and falling cases, respectively, many independent interventions occur at the same time with similar intensity (Figure 1). Therefore, collinearity between interventions was investigated and variables that highly correlate with other variables were removed from models to avoid misinterpretation of results.

Hierarchical Clustering. Despite the removal of some variables due to high collinearity, there is still potential for the significance of NPIs to be overinterpreted due to the implementation of other NPIs at similar times. Therefore, on the basis of the temporal clustering method of Liu et al.,[30] hierarchical clustering of NPIs was performed to assess which interventions are temporally similar. A Gower distance measure was used to calculate the distance between NPIs. When it comes to interpretation of results, the statistical significance of NPIs is interpreted keeping in mind the significance of other NPIs within the same cluster.

Multiple Linear Regression. To determine the effect of NPIs on SARS-CoV-2 infection, multiple linear regression models were established for both Amsterdam and Utrecht, with selected NPIs as independent variables. Two separate models with different dependent variables were created for each city to compare the effect of NPIs on both the SARS-CoV-2 concentration in wastewater and COVID-19 hospitalizations. Given that SARS-CoV-2 prevalence increases and decreases gradually, it was noted that there is a significant serial correlation in residuals for which the linear regression model needs to account. For this, autocorrelation function (ACF) and Partial autocorrelation function (PACF) plots were used to determine the correlation structure of the linear regression models. A generalized least-squares model was fit to the data using maximum likelihood to allow a correlation structure to be incorporated.

Taking into account the time it takes for the population to fully adhere to new rules, and the incubation time, we compared generalized least-squares models with temporal lag periods of 1−21 days (extended to 28 days for the hospitalizations model) to determine the most appropriate delay period. These models were assessed using a comparison of the Akaike and Bayesian information criterion (AIC/BIC) and the log likelihood. Stepwise backward variable selection was subsequently carried out on the chosen linear regression model. The variables selected in this process were validated using stepwise forward variable selection and univariate analysis.

Changepoint Analysis. Changepoint analysis was used in this study to model the temporal patterns of both COVID-19 hospitalizations and N2/CrAssphage between March 2020 and May 2021 in Amsterdam. This analysis aims to identify significant changes in the trajectory of COVID-19 and determine whether the date of these changes can be linked to modifications in the Dutch government's nonpharmaceutical response. For this analysis, piecewise linear regression models were fitted with COVID-19 hospitalizations and SARS-CoV-2 concentration in wastewater as the dependent variable in each respective model, and the date as the independent variable.

BIC was used to choose the most appropriate number of changepoints for both models. Rough dates of peaks and troughs of each time series were selected as priors for the changepoints in both models. Sensitivity analysis was performed by using alternative priors for estimation of the changepoints, and these were consistent. Ultimately, the priors that produced a model with the lowest BIC were used.

The estimated breakpoints and 95% confidence intervals were evaluated against the timing of implementation and relaxation of NPIs by assessing whether any change in COVID-19 NPI policy occurred up to 24 days prior to each changepoint. If NPIs were repeatedly associated with a change in COVID-19 measurements, this would provide strong evidence of the effectiveness of the individual NPI.

■ RESULTS AND DISCUSSION

Collinearity and Clustering. The testing capacity policy and facemask policy have perfect positive collinearity; thus, these variables cannot be considered independent (Figure S1). Therefore, the facemask policy was removed from the linear model analysis, but the interpretation of the testing policy will be considered jointly with the introduction of facemasks if significant. The reduced public transport policy also has a high positive collinearity (−0.78) with the stay-at-home policy and a high negative collinearity (−0.89) with the track-and-trace policy. This is logical considering there is little need for public

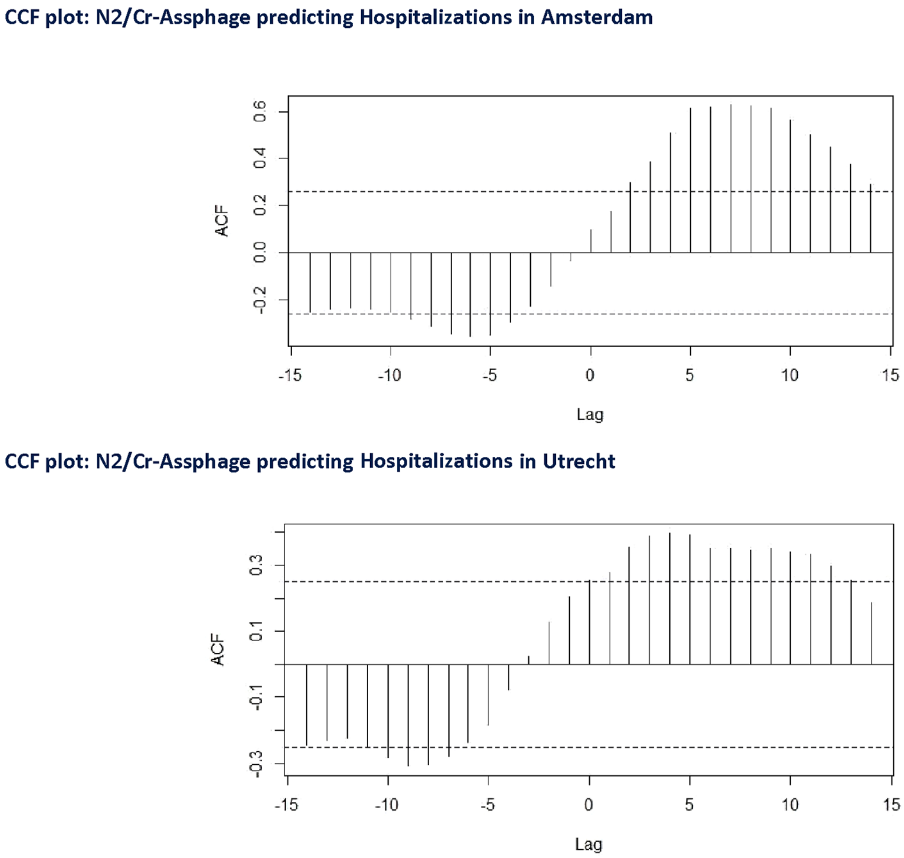

Figure 2. Cross-correlation function (CCF) plots indicating the optimum lag for normalized N2 counts in wastewater to predict hospitalizations in Amsterdam (top) and Utrecht (bottom).

transport if individuals are advised to stay home, and tracing is more crucial when individuals are permitted to travel throughout the country. Due to this, the public transport intervention was removed from the multiple linear regression analysis.

Hierarchical clustering of the remaining NPIs in the analysis produced three significant clusters (Figure S2). This demonstrates that the closure of schools and universities (c1) and stay-at-home (c6) policies are temporally very similar, which is corroborated by a moderately high correlation value between the two (Figure S1). Closure of workplaces (c2), testing (h2), and track and trace (h3) are all within the same cluster and are, therefore, temporally more similar to each other than any other measure. This is understandable between testing and tracing because it is easier to implement more stringent tracing policies if widespread testing is available. Lastly, public event restrictions (c3) and gathering restrictions (c4) form a cluster that is intuitive because events cannot occur while strong gathering restrictions are in place. These clusters will be used for interpretation of final multiple linear models.

Cross-Correlation. Cross-correlation analysis was carried out to consolidate the time delay between the SARS-CoV-2 concentration in wastewater and COVID-19 hospitalizations in both cities. This analysis illustrates that in Amsterdam, wastewater concentrations are highly predictive of hospitalizations occurring 5−9 days later (Figure 2). In Utrecht, wastewater concentrations are most predictive of hospitalization occurring 3−5 days later (Figure 2). This result is corroborated by Peccia et al.,[31] who similarly found that wastewater concentrations preceded hospitalization by 1−4 days.

Shedding of RNA has been found, via nasal swabs, to peak approximately on the day of symptom onset, and previous evidence has suggested there is a median delay of 3−10.4 days between symptom onset and hospitalizations, dependent on age and vulnerability status.[9] It is, therefore, unsurprising that trends observed in wastewater foreshadow hospitalizations. However, no concrete data with regard to fecal shedding have been published. Assuming fecal shedding peaks similarly at symptom onset, the delay period wastewater signal and hospitalization may be longer than suggested in this analysis, but due to weekly sampling of wastewater, the number of measurements that could be compared within the cross-correlation analysis was minimized.

Multiple Linear Regression. For both Amsterdam and Utrecht, ACF and PACF plots of the SARS-CoV-2 concentration in wastewater demonstrate that an autoregression (AR) (1) term is the most appropriate term to use in the generalized least-squares model to account for the correlation structure of residuals (Figure S3). In contrast, an Autoregression moving-average (ARMA) (2,2) term was deduced to be most appropriate for both hospitalization models by trial and error. A comparison of temporal lags using goodness-of-fit measures for the wastewater models in Amsterdam found that a temporal lag of 20 produced the lowest AIC/BIC values and the highest log likelihood (Table S1). The Utrecht model also indicated that a temporal lag of 20 days was preferable to all models with lags of >10 days (Table S2). Therefore, wastewater models with a lag value of 20 were examined

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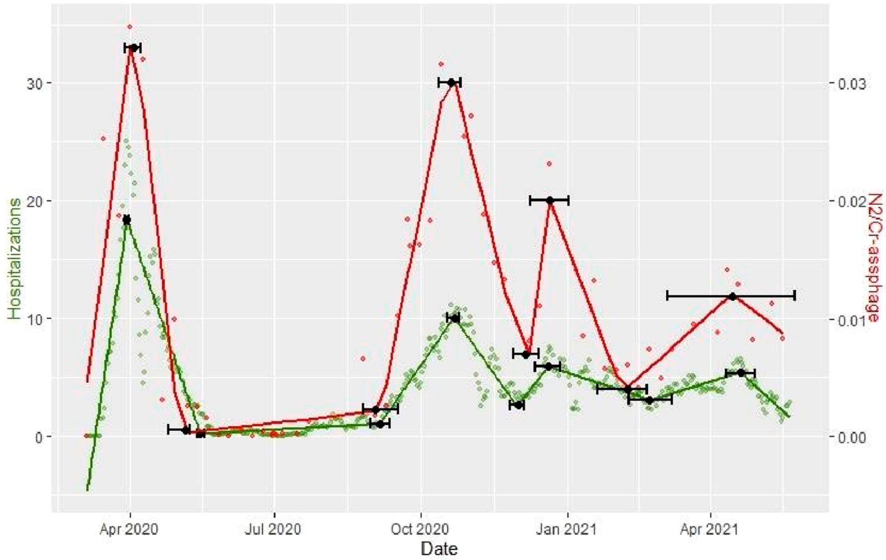

Figure 3. Linear piecewise models fitted to daily hospital admissions in the city of Amsterdam (green line, left *y*-axis) and N2 gene concentrations normalized through CrAssphage concentrations (red line, right *y*-axis). Changepoints are highlighted as black round markers, and the error bar indicates the 95% confidence interval around each changepoint.

Table 1. Backward Variable Selection of Each Linear Regression Model[a]

	c1	c2	c3	c4	c6	h2	h3
Amsterdam, N2/CrAssphage					−/***	+/***	−/**
Utrecht, N2/CrAssphage		−/ns			−/*	+/*	−/*
Amsterdam, hospitalizations		−/***		−/ns			+/***
Utrecht, hospitalizations		−/*		−/ns			+/***

[a]Plus and minus signs indicate the direction of the effect that each NPI has on the dependent variable (left). "ns" indicates the effect is not significant ($p > 0.05$). Asterisks indicate that the NPI is significant: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

further. A temporal lag of 24 days was optimal for both Amsterdam and Utrecht hospitalizations (Tables S3 and S4). These chosen temporal lags corroborate the cross-correlation analysis that suggested a time delay of at least 3 days between the SARS-CoV-2 concentration in wastewater and COVID-19 hospitalizations (Figure 2). These results also suggest an approximate 3 week period between NPIs and observable changes in the wastewater concentration and COVID-19 hospitalizations. Similarly, the work of Stockdale et al.[32] suggests a time delay of up to 3 weeks before impacts of NPIs on COVID-19 cases can be detected.

Because there is a lag between day of infection and the onset date, at the time of the NPI installation, even if they are fully adhered to, there will be first a further increase in the number of infections as a result of these infections that already occurred. In addition, these will infect household members, creating a further lag. Thus, the observable effect is often delayed beyond the incubation period. Additionally, Stockdale et al.[32] also suggested that changes in response to relaxation of measures take even longer to exhibit, so this should also be considered. It is also highly likely that individual interventions will have different time delays based on how much human behavior must change to comply. Closure of schools and universities should have a shorter delay period than most other interventions because adherence to this policy is less flexible and, thus, contact between children and young adults is instantly minimized. This can be observed from the change-point analysis, whereby it took less than a week on four occasions, for this type of intervention to initiate a change in the SARS-CoV-2 concentration in wastewater and COVID-19 hospitalizations (Figure 3).

In terms of backward variable selection of the regression models, it is important to note that while selected variables may demonstrate a correlation with COVID-19 incidence, this does not necessary indication causation. Interpretation as such should be corroborated with existing knowledge. Backward variable selection of the wastewater models both retained the stay-at-home policy (c6), testing capacity (h2), and track-and-trace policy (h3) as shown in Table 1. The stay-at-home policy has a statistically significant negative correlation with SARS-CoV-2 in wastewater in both Amsterdam ($p = 0.0001$) and Utrecht ($p = 0.0143$). The track-and-trace policy also has a statistically significant negative correlation with SARS-CoV-2 in wastewater in Amsterdam ($p = 0.0031$) and Utrecht ($p = 0.0302$). Table 1 demonstrates that the Utrecht model also retained closure of workplaces (c2), which has a negative correlation with SARS-CoV-2 in wastewater but is not statistically significant ($p = 0.0655$). The testing capacity policy has a significant positive correlation with SARS-CoV-2 in wastewater in both Amsterdam ($p = 0.0007$) and Utrecht ($p = 0.0158$). This NPI is in the same cluster as the track-and-trace policy, which has a significant negative correlation, so these results should be treated with caution. Given the perfect positive collinearity with testing capacity (Figure S1), the enforcement of facemasks is also correlated with an increase in the SARS-CoV-2 concentration in wastewater.

A forward variable selection approach for the Amsterdam wastewater model selected the same NPIs, as well as gathering

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restrictions (c4), which has a positive correlation with SARS-CoV-2 in wastewater. However, this variable is insignificant ($p = 0.5427$), so backward variable selection is preferred. A forward variable selection approach for the Utrecht wastewater model included only stay at home (c6). Univariate analysis supports the conclusion that the stay-at-home policy has a significant negative correlation with the SARS-CoV-2 concentration in wastewater in both cities (Table S5). Univariate analysis also indicates that closure of schools has a significant negative correlation with wastewater load in Utrecht (Table S5). Given that the closure of schools and the stay-at-home order are within the same cluster, it is possible that the effect of school closures is being masked by the stay-at-home policy (Table 1). Regardless, there is strong evidence to suggest cluster 1 significantly decreases the SARS-CoV-2 concentration in wastewater.

Within the hospitalization models for both cities, backward and forward variable selection methods both selected closure of workplaces (c2), gathering restrictions (c4), and track-and-trace (h3) policies to include in the final model (Table 1). The closure of workplaces has a significantly negative correlation with COVID-19 hospitalization in both Amsterdam ($p < 0.0001$) and Utrecht ($p = 0.0351$). The gathering restriction policy also has a negative correlation with hospitalizations in Amsterdam ($p = 0.1065$) and Utrecht ($p = 0.1212$), but it is not a significant factor in either model. However, it is a highly significant factor within univariate analysis of hospitalizations (Table S5). This univariate significance may be an effect of some collinearity (0.58) with workplace closures (Figure S1), given its significance in both reduced hospitalization models and univariate analysis (Table S5). Lastly, the track-and-trace policy has a significant positive correlation with hospitalizations in Amsterdam ($p < 0.0001$) and Utrecht ($p < 0.0001$). Again, the track-and-trace policy is in the same cluster as closure of workplaces (c2), so this result should be treated with caution. Univariate analysis came to a similar conclusion whereby track and trace (h3) and testing (h2) have significant positive correlations with COVID-19 hospitalizations in both cities (Table S5).

While the results between Amsterdam and Utrecht are consistent, the results of the Utrecht model are generally associated with wider 95% confidence intervals and, thus, higher associated uncertainty of conclusions (Figures S4−S7). This may be because the city has almost half of the population of Amsterdam; therefore, larger random variation is associated with wastewater measurements, and small numbers of daily hospitalizations can make effects of measures less noticeable.

Numerical scaling of interventions may lead to misinterpretation of results given that intensity levels are not equally spaced in reality. While this approach was not possible within multivariate analysis, univariate analysis of interventions using labeled interventions instead was explored to validate results (Figures S8−S11). This analysis supports the results presented above whereby stay-at-home (c6) and track-and-trace (h3) policies reduce the SARS-CoV-2 concentration in wastewater (Figures S8 and S9). Additionally, Figures S10 and S11 support the conclusion that closure of workplaces (c2) and gathering restrictions (c4) reduce COVID-19 hospitalizations. Figures S9 and S10 also indicate that closure of schools (c1) reduces hospitalizations. It should be noted that many NPIs did not have four levels of implementation intensity, or if they did, the distribution of data points at each level was highly skewed, which decreased the level of confidence of estimates at some levels. This will only improve as more data are collected.

This analysis provides evidence to suggest that a stay-at-home order significantly reduces the SARS-CoV-2 concentration in wastewater. However, this effect was not reflected in the linear regression models of hospitalizations in either city. After inspection of the two instances in which the stay-at-home order was enforced, wastewater and hospitalization measurements are much less closely aligned in December 2020 than in April 2020. Hospitalizations experienced a much shallower increase and subsequent decrease during the second wave. This could be a factor in why the stay-at-home policy does not appear to significantly reduce hospitalizations within regression models.

The misalignment during the second wave between wastewater signal and hospitalizations may be because more young individuals became infected during the second wave and the severity of the disease is reduced in young individuals. Therefore, there was a high level of circulation of SARS-CoV-2, but a smaller proportion of cases required hospitalizations. The reduced severity in younger populations is a possible explanation for why the closure of schools was insignificant in both hospitalization models but was significant in univariate analysis of wastewater models. However, this measure was also instigated to reduce transmission between children and adults, not only to reduce circulation among children.

Another measure that presented strong evidence of its efficacy is the closure of workplaces, which includes retail, hospitality, and leisure establishments, as well as offices. The closure of workplaces was significant in three of the four reduced linear regression models and had a negative effect in all three (Table 1). From univariate analysis, this measure seemed to be a particularly significant factor in reducing hospitalizations in both cities, which could be explained by the age demographic of the people that this measure affects. This result is in line with many other NPI studies whereby 86% found it to be an effective measure.[33] The closure of workplaces has sparked much debate due to the increasing strain on the economy. Many governments, including the Dutch government, introduced furlough schemes to subsidize the loss of income to businesses and staff, but this is not a sustainable long-term solution. The flexibility of this approach as well as the notable reduction in hospitalizations makes this a very effective measure. However, this interpretation is reasonable only while a furlough is still in place; once this scheme ends, adherence and the subsequent effectiveness are likely to decrease substantially.[34]

Sufficient evidence was not found to demonstrate the effect of gathering restrictions (c4) on the SARS-CoV-2 concentration in wastewater for either city. Categorical univariate analysis (Figure S9) even suggested a positive correlation between this measure and the SARS-CoV-2 concentration in wastewater in Amsterdam, particularly as measures increased from "no action" to "recommended intervention". This is in contrast to the results of Brauner et al.,[4] who used data from only the first wave in early 2020. It is possible that the effectiveness of this measure has since decreased as adherence has waned.[34] Gathering restrictions were found to have a negative effect in the linear regression model of hospitalizations in both cities, although the effect is insignificant. The inclusion of gathering restrictions in the reduced hospitalization models may be because hospitalizations reflect the situation among a subgroup of the population (mostly elderly

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and more vulnerable people), while wastewater is generalized. Compliance with gathering restrictions may play a role here as it has been demonstrated that older individuals have followed the restrictions more stringently, possibly out of fear of COVID-19 or respect for rules.[35] Regardless, gathering restrictions are an insignificant factor in all reduced models, which suggests they have a limited impact on virus circulation. Similarly, no substantial evidence in this analysis can be found to support the effectiveness of public event bans (c3) in any model. However, the lack of evidence in these two cities does not mean that this measure is ineffective everywhere. There will be differences in the number of public events and the response to recommended cancellations in different localities, so this should be considered when interpreting these data.

While the track-and-trace policy is associated with a decrease in the SARS-CoV-2 concentration in wastewater in both cities, this is not reflected in COVID-19 hospitalizations whereby a positive association is found (Table 1). Increased testing and, thus, also the enforcement of face masks due to the collinearity of the measures (Figure S1) were also found to have a positive association with both the SARS-CoV-2 concentration in wastewater and COVID-19 hospitalizations. It is likely that these positive associations are not the product of a causal relationship but may be an effect of the parallel increase in testing capacity with the increase during the second wave in the winter. It may also be the result of negative collinearity with other measures that have been relaxed.

Changepoint Analysis. As described in the methodology, piecewise linear regression models were fitted to time series of the SARS-CoV-2 concentration in wastewater and COVID-19 hospitalizations in Amsterdam. Both time series had high adjusted R^2 values of ∼0.85 when fitted with eight changepoints. Figure 3 highlights the temporal similarity in changepoint location between the two COVID-19 measures, which indicates that eight events have occurred, causing significant changes in COVID-19 hospitalization and wastewater signal. Despite expectations, estimated dates of changepoints in the wastewater measurements do not consistently precede hospitalization changepoint dates. However, the date of the lower 95% confidence interval of wastewater measurements occurs before the date of the lower 95% confidence interval for hospitalizations on almost every occasion (Table S6).

The two major changepoints and subsequent declines of COVID-19 in late March and early April 2020 and late December 2020 (Figure 3) cannot be attributed to a single intervention but instead are the result of a combination of multiple interventions. Between 1 and 3 weeks before the first changepoint in both models (March 30 and April 3 for wastewater and hospitalization measurements, respectively), all public events were canceled, all schools and universities were closed, some workplaces were closed, full gathering restrictions were implemented, and a stay-at-home order was introduced (Figure 1). Similarly, a combination of interventions came into play on December 15 in response to rapidly rising infections, including full closure of schools and universities, full closure of non-essential workplaces, and a stay-at-home order (Figure 1). This led to a steep decline in both circulation of SARS-CoV-2 in wastewater and hospitalizations after December 21.

While this analysis does demonstrate that the enforcement of both stay-at-home orders coincides with subsequent steep declines, it is easy to overestimate the impact of this measure. This is because, on both occasions, the stay-at-home order has been implemented in conjunction with full closure of schools and universities and increased closure of workplaces. It is, therefore, very difficult to evaluate this measure separately. Other studies have considered this NPI only as an additional measure to others already implemented and found that it had a limited additional effect.[4] It has been suggested that compliance with the stay-at-home policy was reduced in December 2020 compared to April 2020, when people were still substantially more afraid of COVID-19.[35] This poses the question of whether an intervention can be considered effective if the measure is too restrictive that the general public can no longer adhere to it, now that fear of COVID-19 has diminished. Adherence is likely to become more fragile as fear diminishes and immunity increases.[34,35]

Between the September 5 and 6, 2020, both measurements experienced a changepoint whereby the level SARS-CoV-2 in the community began to increase, particularly the concentration in wastewater (Figure 3). Within 24 days before this time point, only one relaxation of NPIs occurred and this was the reopening of schools with distancing measures at the start of September after school holidays (Figure 1). This event increases the likelihood of transmission outside the home between children and young adults, but also within the home between children and potentially vulnerable adults, thus explaining the rapid increase in both the wastewater concentration and hospitalizations.

Again, when schools closed for school holidays around October 17, both the SARS-CoV-2 concentration in wastewater and hospitalizations experience a peak on October 19 and 22, 2020, respectively (Figure 3), and quickly decreased. During this week, the transmission was briefly cut off, which drastically reduces the number of cases. Further evidence in support of school closures is provided after the February break when the curfew was still in place. The trajectory of both the SARS-CoV-2 concentration in wastewater and COVID-19 hospitalizations is estimated to have changed to an increasing state on February 12 and 23, respectively (Table S6), only once schools had reopened on February 7, 2021. These results are consistent with a recent review in which 58% of studies found the closure of schools to be associated with a reduced number of cases.[33]

Given that hospitalizations experience shallower increases when schools reopen, it could be argued that numbers of cases among children are less concerning because symptoms are usually mild and the strain on healthcare facilities is less pronounced. Additionally, the closure of schools can negatively affect the economy and the operations of healthcare facilities. Therefore, in the future, it may be sensible to suggest that schools should not be closed except in the most extreme cases due to the lack of a strong association with hospitalizations. It is important to note that the closure of different levels of schools, including preschool, primary, secondary, and higher education, could not be compared given the structure of the OxCGRT data.[28]

At the beginning of December 2020, the SARS-CoV-2 concentration in wastewater and COVID-19 hospitalizations began to increase again at a rate similar to that in September (Figure 3). The only NPI to be linked to this changepoint is the reopening of workplaces on November 22 (Figure 1). The return to work for some individuals would have increased the risk of transmission. Therefore, the subsequent increase in COVID-19 cases in December could be attributable to the reopening of workplaces. Together with the contribution to the two major changepoints in April and late December, this

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analysis corroborates the evidence from the linear regression models that the closure of workplaces is a particularly effective policy. However, another factor that is likely to have contributed to this sharp increase in cases over the winter is worsening weather. It is well-known that respiratory diseases are seasonal and infections are more common in the winter months. Winter weather should, therefore, be considered to be an additional driver for increasing numbers of cases between September 2020 and February 2021.

The last changepoint was estimated to occur April 15 and April 20, 2021, for the SARS-CoV-2 concentration in wastewater and COVID-19 hospitalizations, respectively (Table S6). However, the 95% confidence interval of the wastewater changepoint is wide, spanning more than 5 weeks on either side, and extending far beyond the hospitalization changepoint, so this estimation is weak. No change in NPIs occurred during the evaluation period for either measure, so the subsequent decline in COVID-19 prevalence cannot be credited to any NPI. However, vaccination of the population against COVID-19 began on January 6 in The Netherlands, and the level of vaccination has slowly increased since.[29] Therefore, it is possible that this decreasing rate in both time series can be attributed to increasing immunity to COVID-19. The rollout of the vaccination began to pick up speed only in March, so limited vaccination data were available at the time of collection. Data on vaccinations and seropositivity results at the municipal level could also not be extracted. However, future research could extend this analysis to consider these factors if immunity data at this finer spatial scale become available.

CONCLUSION

The findings of this work show that the estimated changepoints of SARS-CoV-2 in wastewater throughout the pandemic are associated with much higher uncertainty than hospitalization because of the infrequency of wastewater measurements. Often samples were taken weekly, but frequently gaps between measurements exceed 10 days, which is likely to have reduced the accuracy of changepoint estimation. To consolidate the delay between measurements and provide accurate changepoints, higher temporal granularity of wastewater sampling is needed. This will, however, increase costs associated with wastewater surveillance, but the potential for WBE to predict hospitalization trends earlier than is currently possible could be considered invaluable.

While this study benefited from a long time period, the transmission rate and, therefore, the relationship between cases and hospitalizations are likely to have changed over the year due to the dominance of different SARS-CoV-2 variants. This study did not take into account data from different variants measured in wastewater because at the time of analyses these were not available. However, it is now possible to distinguish among variants circulating in wastewater,[27] so this could be accounted for in future work by adding in an interaction term between NPIs and a variable that represents the change in variants. Future work would also hope to extend this research to compare different countries. WBE is not implemented universally to the scale that it currently is in The Netherlands, so comparisons between countries are limited. While some countries, such as the U.K., have used WBE to monitor SARS-CoV-2,[22] there is a chance of interlab variation in sampling methodology and qPCR design that can make use of data from different laboratories difficult. Standardized protocols have been suggested,[36] which would allow for comparative studies of WBE.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.2c00071.

Figures S1−S11 and Tables S1−S5 (PDF)

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Notes

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